

APPENDIX B

LABELING CONSIDERATIONS FOR PRODUCT PACKAGE INSERT FOR *IN VITRO* DIAGNOSTIC DEVICES THAT UTILIZE CYTOGENETIC *IN SITU* HYBRIDIZATION TECHNOLOGY FOR THE DETECTION OF HUMAN GENETIC MUTATIONS (CONSTITUTIONAL AND SOMATIC)

Assure that the labeling complies with Section 502(a) of the Act that the directions for use are not false or misleading and section 502(f)(1) of the act that directions for use are adequate. (Section 201(n) of the Act defines misbranding due to misleading labeling.)

Follow 21 CFR 809.10 for the requirements for labeling of *in vitro* diagnostic products. As stated in 21 CFR 801.119, this will meet the regulations for compliance with the Section 502(a) of the Act, Section 502(f)(1) of the Act and 21 CFR Part 801, Labeling.

Do not make unproven claims for clinical significance in the package insert (PI).

The following are details for some of the points in the above statutes and regulations that should be addressed in the PI.

A. PROPRIETARY NAME AND ESTABLISHED NAME

State common or usual name, if any.

B. INTENDED USE OR USES:

Provide a concise description of information about the product, including but not limited to:

1. Essential Information:
 - a. State whether the assay is quantitative or qualitative.
 - b. Identify analyte being detected, i.e., identify the target sequence(s), locus/loci, chromosome region, or whole chromosome being detected (e.g., chromosome 21 alpha-satellite; abl/bcr breakpoint region, etc.)
 - c. State methodology/technology employed by the device.
 - d. Special instrumentation requirements.

- e. Identify all specimen types/matrix(ices) (tissue and cell type) used by the test methodology(ies).
- f. Indicate whether culture, direct preparations, or both are required.
- g. State cell cycle stage for analysis (metaphase and/or interphase, G1 vs. G2)
- h. Target population and indications for use

Define the target population to be tested using the device. State whether for screening; monitoring; carrier detection; aid in the diagnosis of ...; as an adjunct to ...; as a "stand alone"; whether for prenatal or postnatal use; etc.

2. Clinical significance:

State concisely the clinical significance of the device. If the clinical significance statement is lengthy or complicated, create a separate heading entitled "Clinical Significance".

Specify what user qualifications/special training are needed to perform the assay and/or interpret results.

3. Conditions for use:

Describe any special applications of the device or specific contraindications or indications for use not addressed in the Intended Use statement.

These may be addressed further elsewhere in the PI, e.g., in either the Summary and Explanation or Limitations section.

For PMAs, the Intended Use statement in the PI must match exactly the Indications For Use statement in the Summary of Safety and Effectiveness Document.

C. SUMMARY AND EXPLANATION OF THE TEST

- 1. Provide more detailed discussion of clinical indications/significance/utility that was stated briefly under intended use and include relevant information described in section I. of the guidance document.
- 2. Provide a historical summary of methods used to detect the disorder

with appropriate literature references and include a bibliography at the end of the PI.

3. Discuss the special merits and limitations of device's methodology and the rationale for using ISH.
4. Discuss whether other confirmatory/follow-up testing (e.g., conventional karyotyping), clinical evaluation, etc., are recommended.

D. PRINCIPLES OF THE PROCEDURE

Provide a description of the test methodology as discussed in section **II.A.** of the guidance document.

Characterize detection probe, detection system, etc., as discussed in section **II.B.1. & 2.** of the guidance document.

E. REAGENTS/KIT COMPONENTS

Characterize all reagents/components provided in the kit.

1. Concentration (DNA/RNA, etc. in ug/ml).
2. Biological source (e.g., bacteria, bacterial plasmid, bacterial phage, yeast, etc.)
3. Provide storage instructions and stability claims (both unopened and opened/reconstituted).
4. State any known indications of instability and/or deterioration.
5. Provide instructions for reconstitution, mixing, dilution, etc.
6. Include appropriate safety precautions/warnings statement concerning protection from physical hazards and biohazardous material and proper disposal procedures for such materials.

Include the following statement in the package insert: "For *in vitro* diagnostic use".

F. SPECIMEN COLLECTION AND HANDLING

Include a description of:

1. Type of specimen (tissue and cell type) to be collected, e.g., peripheral whole blood (for studying T-lymphocytes), bone marrow, amniotic fluid (amniocytes) skin biopsy (fibroblasts), etc.
2. Collection procedure and precautions.
3. Amount of specimen required, both optimum and minimum.
4. Known interfering substances or conditions specific to specimen collection and preparation.
5. Procedure for storage, handling and transport of specimen including additives, preservatives, etc. for the protection and maintenance of specimens; and length of stability of the specimen for the recommended storage temperature/condition requirements.
6. State storage conditions for slides and/or fixed cell pellets.

G. PROCEDURE: Directions for Use

Provide step-by-step protocol/outline of assay procedure to address: specimen preparation and processing (culturing, harvesting, etc.); denaturation procedure for target and probe (double stranded); hybridization reaction; signal detection; and analytical requirements for counting, analyzing, and interpreting results.

1. List materials needed, not provided (Note: FDA regulated materials must be FDA cleared/approved).

Provide information on microscope requirements and characteristics (calibration) for ISH; provide information regarding the type of microscope required, e.g., light source, filters, confocal, and eye piece specifications. Describe any special specification/requirements and any materials that are contraindicated for use. For example, certain microscope eyepieces reportedly have compounds on their lenses which will autofluoresce after prolonged usage such that signal detection is reduced.

2. Describe amount of reagent(s), time, temperature tolerance, light source, etc. needed to run the assay.
3. State the stability of the final reaction product.

H. QUALITY CONTROL (QC)

Provide adequate directions for performing QC procedures that include, but are not limited to, the following:

1. Designate specimens or commercially available products that could appropriately be used for positive and/or negative control to be tested with the same protocol as patient specimens for control of probe analytic performance. Include recommended levels, if appropriate. Consider whether some type of informed consent is required for use of other patient's samples as controls.
2. Directions for performing quality control.
3. Recommendations for testing frequency and placement of quality control material within run and run-to-run.
4. Directions for interpretation of results of quality control samples (satisfactory limits of performance).
5. Description of remedial action to be taken when control results fail to meet criteria for acceptability. Provide troubleshooting instructions.

Conclude with a statement similar to the following: "If controls do not behave as expected, assay results are invalid."

6. Recommendations for quality control parameters other than positive and negative controls, if appropriate. Provide alternate QC recommendations to control all aspects of the procedure to assure the safety and effectiveness of the device and to control the risk for false positive and false negative results.

Stress the importance of maintaining strict adherence to operational procedure parameters to assure specificity of the hybridization condition and that failure to adhere to such controls may result in false positive and false negative results.

8. Provide trouble shooting section

Describe potential problems that may be encountered, probable cause, and actions to be taken to correct the problem.

- a. Address special precautions necessary to control for variability of conditions of each step of the procedure, e.g., sample preparation, denaturation, hybridization, and probe detection. For example, stress the importance of monitoring equipment, such as water bath temperature, that has the potential to induce critical variables.

- b. List points useful for improving "precision and accuracy" (e.g., stringency requirements).
- c. Discuss steps necessary to prevent non-specific hybridization.
- d. Describe specific conditions or components (reagents) needed to minimize effects of cross-hybridization and background noise.

I. INTERPRETATION OF RESULTS

When indicated, the following issues should be addressed:

1. State criteria (procedure/calculations) necessary for interpretation of test results (including positive, negative, equivocal/borderline/indeterminant results).
2. Specify criteria for scoring the number of probe signals. State how many cells must be examined for reliable test reporting. The recommendations should be based on the degree of confidence necessary.
3. Address need for objectivity of microscope scoring methods and how this objectivity may be accomplished.
4. For chromosome enumeration products, discuss the ability/inability of the device to distinguish between true mosaicism and artifact due to variable hybridization in the cell preparation. Indicate whether standard cytogenetic analysis is required to confirm/resolve this issue.
5. Discuss whether differential signal intensity between chromosomes of a pair or between cells within a given specimen will effect interpretation of results.
6. Consider the risk for false positive results due to cross-hybridization to other chromosomes/nucleic acid sequences in non-banded preparations.
7. Discuss how to distinguish G1 cells from G2 cells for interphase analysis.
8. Address how/whether genetic heterogeneity, genetic polymorphisms, or other genetic mechanisms impact on test interpretation. Genetic polymorphism applies, primarily, to single gene defects although satellite (repetitive) DNA may show variable intensity of the signal because of variability in the copy number of the target DNA.

9. State whether any other tests (e.g., conventional karyotyping, chromosome banding, etc.) are recommended to confirm results or whether other individuals should be tested to determine the heritable or spontaneous origin of the relevant mutation. For example, in cases of microdeletions, a proportion of patients with apparent deletions arise from parental chromosome rearrangements. Therefore, parental chromosome studies using sequential banding and ISH will be necessary in any probe negative cases.

10. Include recommendations of the terminology and appropriate nomenclature to use for test reporting.

J. LIMITATIONS

List important test limitations and all known contraindications, with references.

1. Include limitations for any special applications or specific contraindications or potential known indications for use of the device for which FDA clearance/approval is not sought/granted.
2. Describe any cross-hybridization (e.g., stringency dependent) and characterize cross-hybridizing sequences (chromosomal location, etc.).
3. Describe assay interferences/interfering substances.
4. State whether test results should be used in conjunction with other clinical, history, or laboratory information for diagnosis.
5. Describe factors that may result in false positive and false negative results. For example, Trisomy 21 translocation (approximately 5 percent of cases) may not be detected with interphase analysis due to the choice of probes. If centromeric probes are used, two signals will be observed whereas non-centromeric, distal probes will yield three signals.

K. EXPECTED VALUES

Provide expected values (reference ranges) obtained using the device from studies of appropriate populations:

1. Describe the protocol used to establish expected values including the number and types of specimens used for each appropriate study populations.

For metaphase analysis of centromeric probes, the data should distinguish

between absence or presence of signal on one or both chromosomes of a pair; for non-centromeric probes, distinguish between absence or presence of signal on one or both chromatids of a pair of chromosomes (except for Y). For interphase analysis, report the proportion of cells with 0, 1, 2, 3, 4, etc., signals per cell and the proportion of unreadable cells. Include the number of cells analyzed.

2. State and reference the expected prevalence/frequency of the mutation or disease in the target population(s). Discuss any variability in prevalence between populations and describe factors that impact on such variability (e.g., ethnic differences).
3. Define limits of mosaicism detection and guidance to users for establishment of safe parameters of rates of signal detection around which the lab builds its own baseline data (i.e., quality control for interpretation of results).
4. For acquired abnormalities, there must be sufficient data on baseline rates of detection to safely distinguish that proportion of cells in which one or no signal is seen so that a low grade monosomy (minimal residual disease or small clone) will not be misinterpreted (this is of lesser concern when probes for site specific rearrangements are used).
5. State that each laboratory should determine their own baseline expected values.

L. PERFORMANCE CHARACTERISTICS

Summarize the data upon which the performance characteristics of the test are based, e.g., analytic sensitivity and specificity, precision (repeatability and reproducibility), clinical/diagnostic sensitivity and specificity, as suggested in section III. of the guidance document. This discussion should include a concise description of the study protocols used to evaluate the test's performance.

For interphase analysis, define the proportion of all clinical/genetic cases not detected by the device, i.e., proportion of false negatives. The calculation is based on current data frequency of structural abnormalities which would go undetected with this method. For prenatal, this must be addressed at any stage of gestation at which testing is done. Since this can vary with clinical indications for patient testing, data will need to be stratified by indication.

If positive and negative predictive values are estimated, this requires knowledge about the prevalence of the disease in the population sampled. Relate the relevance of any

predictive values for a particular population and point out that this will not, necessarily, be applicable to determining the probability of a particular state of nature for a given individual.

M. Bibliography

Include a bibliography of references cited in the text and any other references related to the subject matter.

We recommend the following document for uniformity of the bibliography style: International Committee of Medical Journal Editors. **SPECIAL REPORT**, Uniform Requirements for Manuscripts Submitted to Biomedical Journals. *New Engl J Med* 1991;324:424-8.

APPENDIX C

GLOSSARY FOR REVIEW CRITERIA FOR *IN VITRO* DIAGNOSTIC DEVICES THAT UTILIZE CYTOGENETIC *IN SITU* HYBRIDIZATION TECHNOLOGY FOR THE DETECTION OF HUMAN GENETIC MUTATIONS (CONSTITUTIONAL AND SOMATIC)

ACCURACY The property of an estimate in which it is equal to the actual value on an average. The opposite of bias.

ALLELE Alternate form of a gene at a given locus.

ALLELIC HETEROGENEITY Multiple allelic mutations at a given locus. Sometimes referred to as "allelic diversity".

ANEUPLOID A chromosome number that is not an exact multiple of the haploid number.

ANALYTE A substance or constituent for which the laboratory conducts testing.

ANNEALING Hybridization of two complementary strands of nucleic acids (DNA or RNA) as in the hybridization of a probe with the target DNA/RNA.

BIAS The difference between a known value and the long run average of repeated sample tests performed to estimate that known value. If that difference is zero, the test procedure is unbiased (i.e., accurate).

CALIBRATOR/CALIBRATION MATERIAL (Synonyms: Standards (NCCLS LA-18-P); verifier material) Material, solution, or lyophilized preparation appropriate for the methodology and, if possible, traceable to a reference method and reference material of known value. The values or concentrations of the analytes in the calibration material are known within limits that have been established during its preparation, and confirmed in use. (Thiers NCCLS)

CARRIER A individual (usually unaffected) who is heterozygous for a normal gene and a mutant gene at a given locus in the diploid state.

CFR Code of Federal Regulation

CLIA '88 Clinical Laboratory Improvement Act of 1988

COMPARISON OF METHODS Statistical procedure that is based on data gathered

from the paired analysis of the same specimens by two different methods. When evaluating substantial equivalence between a new device and a predicate device, the performance of each device is, ideally, compared to a diagnostic endpoint or to a well defined reference method, sometimes called a "gold standard".

CONFIRMATORY TEST More specific than a screening test. Used to confirm screening test results for purposes of patient management and care. (see diagnostic test)

CONSTITUTIONAL MUTATIONS Germ line mutations that are present at conception and are, theoretically, found in all cells of the organism.

CONTIGUOUS GENES: Genes that map next to each other on a given chromosome.

CRYPTIC TRANSLOCATION A sufficiently subtle translocation event involving telomeric ends of chromosomes such that it would not be reliably detected at the 400-500 band stage of resolution. [Ledbetter DH: Minireview: Cryptic Translocations and Telomere Integrity. Am J Hum Genet 51:451-456, 1992.]

CONTROL A material, cell line, solution, pool of collected specimens, etc. designed to be used in the process of quality control to assess the validity of the test. The concentrations of the analytes of interest in assayed control material are known within limits ascertained during its preparation, and confirmed in use. (Thiers NCCLS) The concentration and limits of the analytes/cells/targets of interest in unassayed controls are not provided by the manufacturer; laboratories must determine the values of unassayed controls.

CONTROL PROCEDURES Control procedures are performed on a routine basis, in order to monitor the stability of the method and test system, and indirectly assess the accuracy and precision of patient test results (§493.1213 And §§493.1223 - 493.1285. Specialty and subspecialty control procedures are specified under §493.1223. (CLIA §493.1202)

CUT-OFF POINT The reporting level in a quantitative or semi-quantitative test designed to distinguish between two qualitative states (e.g., positive vs. negative; disease vs. non-disease; one genetic state vs. another; reactive vs. non-reactive, etc.). A cut-off should be determined using a sample of observations on subjects for which the diagnostic status is established and should come closest to achieving a certain balance between sensitivity and specificity. The balance to be achieved depends the intended use of a device and the prevalence of the disease/state in the target population.

DENATURATION Separation of double-stranded nucleic acid (hybridized strands) to

a single stranded state with minimal secondary structure. Denaturation is accomplished by heating, increasing pH, or adding agents such as formamide or urea. Denatured NA strands are available for hybridization with a probe or primer.

DEVICE The FDA uses the Code of Federal Regulations definition of a medical device to include "*in vitro* diagnostic products". This includes those reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for examination of specimens taken from the human body. These products are devices as defined in section 201(h) the Federal Food, Drug, and Cosmetic Act, and may also be biological products subject to section 351 of the Public Health Service Act. (CFR 21 Part 1 Subpart A) (Synonyms: assay, IVD, test)

DIAGNOSTIC TEST A test applied to a specimen from an individual at increased risk for a particular disorder/genetic state, e.g., due to a positive family history or symptoms. The test is intended to measure or predict the diagnostic endpoint of interest, e.g., clinical outcome (phenotype) or genetic status (genotype).

EFFECTIVENESS There is reasonable assurance that a device is effective when it can be determined, based on valid scientific evidence, that in a significant proportion of the target population, the use of the device for its intended use and conditions of use, when accompanied by adequate directions for use and warnings against unsafe use, will provide clinically significant results." [21 CFR 860.7(e)(1)]

ERROR Deviation from truth. Error can be due to bias and/or to imprecision. (Thiers NCCLS)

EXPECTED VALUES The range of values established by the manufacturer or medical literature in samples from defined populations, asymptomatic normal individuals, individuals with certain disorders/states of interest, etc.

FALSE NEGATIVE TEST RESULT Negative test result obtained for subjects who are truly positive for the disorder/state of interest.

FALSE POSITIVE TEST RESULT Positive test results obtained for subjects who are truly negative for the disorder/state of interest.

FDA Food and Drug Administration

FDA/OC FDA's Office of Compliance

FDA/CDRH FDA's Center for Device and Radiological Health

FDA/ODE FDA's Office of Device Evaluation

FFD&C The Federal Food, Drug, and Cosmetic Act of 1932

GENETIC DIAGNOSIS The genetic status of an individual for a trait or disorder of interest. The diagnostic endpoint of interest may be clinical outcome (phenotype) or genetic constitution (genotype).

GENETIC HETEROGENEITY Different genetic mechanisms (e.g., nonallelic mutations) responsible for a given phenotype or very similar phenotype.

GENOTYPE The genetic constitution of an individual.

GOLD STANDARD METHOD A definitive method accepted as revealing the true status (e.g., disease or non-disease) of a sample, of use in evaluating the accuracy of a new method applied to the same samples as the gold standard method. If no definitive method is available, a reference method is sometimes used for method comparison but this will lead to biased estimates.

HHS The Department of Health and Human Services, or its designee. "Secretary" is used in 21 CFR refers to the Secretary of Health and Human Services.

HETEROZYGOUS The presence of alternate alleles at a given locus. Any one individual has only two possible alleles at a given locus in the diploid state.

HOMOLOGY (between nucleotides) The degree of base pair matching between the target and the probe.

HYBRIDIZATION Base pairing of complementary strands of nucleic acid by hydrogen bond formation to produce an RNA-DNA or DNA-DNA hybrid.

HYBRIDIZATION EFFICIENCY [Seelig SA, Vysis, Inc., Personal communication, 1/31/96]

(1) Cellular basis:

$$\frac{\text{Total number of cells with one or more signal}}{\text{Total number of cells examined}}$$

(2) Target basis:

Given that normal cells have two signals, then

$$\frac{\text{Total number of cells with two signals}}{\text{Total number of cells with at least one signal}}$$

This percentage will need to be corrected downward slightly by the frequency of three signaled cells due to the fact that occasional two signaled cells will arise due to small amounts of non-specific binding. This correction allows for a conservative estimation of hybridization efficiency and specifically assumes that three signaled cells arise solely from non-specific hybridization. If there is a biological basis (extra DNA target is available) for 3 signaled cells, then this correction is not necessary.

The first definition may be useful to assess sample preparation procedures while the second definition may be the best for characterization of probe efficiency.

IDE Investigational Device Exemption under section 520(g) of the Act and Part 812 and 813.

IMPRECISION The property of estimates in which repeated measurements are not equal to the same value.

INTENDED USE This refers to how the test is to be used, e.g., diagnosis, screening, monitoring, etc.; adjunct to existing methods or stand alone for test reporting; the matrix of the specimen, e.g., serum, urine, etc.; and the setting where the test will be performed, e.g., a moderate complexity laboratory vs. a high complexity laboratory.

IN SITU HYBRIDIZATION (ISH) Nucleic acid hybridization applied to cells.

INVESTIGATIONAL DEVICE Requires labeling: "For Investigational Use Only. The performance characteristics of this device have not been established." Use of the device is presumed to be for the collection of data to support a claim for intended use with clinical utility. It also assumes that the user has an Institutional Review Board (IRB) approval which may require informed consent. If test results are to be used for patient care without confirmation by another diagnostic test or procedure, an approved Investigational Device Exemption application is required.

KIT All components of a test that are packaged together.

LOCUS The chromosomal position at which a gene/DNA sequence resides.

LIMITS OF DETECTION For devices making a quantitative claim, the minimum detection limit is the lowest concentration of an analyte (e.g., target/allele/mutation) that is reliably detectable by the specified analytical method. (See reliability). For ISH, this may apply to the smallest concentration of cells with the target of interest that can

be detected with the device, e.g., minimum residual disease in hematological disorders.

MATRIX The milieu (e.g., specimen type) containing the analyte (e.g., target/allele/mutation) of interest in a control material or patient specimen submitted for analysis.

MDA '76 Medical Device Amendments of 1976

MONITORING TEST Intended to assess response to therapy or detect residual or recurrent disease for previously diagnosed disorders.

MOSAICISM The occurrence together of two or more genetically distinct cell lines in an individual; results from a postzygotic event.

NULL HYPOTHESIS A statistical hypothesis provides the statistician with a specific quantitative value for some parameter (e.g., sensitivity) and provides the biologist with a biologically meaningful state. Null hypotheses are usually ones that an investigator hopes to be able to reject on the basis of investigational data, e.g., if an investigator hopes to show that a new method performs better than a comparative method, the null hypothesis would be that the new method's performance is no better than that of the comparison method. When an investigator hopes to show equivalence of two methods, the null hypothesis will be accepted as being true.

PERFORMANCE CHARACTERISTIC A property of a test that is used to describe its quality, accuracy, precision/repeatability, reproducibility, analytical sensitivity, analytical specificity, reportable range, reference range, or other relevant characteristics of a test as revealed by its use in the laboratory.

PHENOTYPE The observable characteristics of an individual that result from the expression of the genotype.

POLYMORPHISM The occurrence of two or more alternate alleles at a given locus such that the most common has a frequency of less than 0.99. A polymorphism in a DNA sequence is referred to as a restriction fragment length polymorphism (RFLP); it is detected when the DNA is digested with a particular restriction enzyme that yields different fragment lengths.

PRECISION The property of estimates in which repeated measurements (under the same conditions) will give the same value.

PREDICATE DEVICE A legally marketed device intended for in-vitro diagnostic use. This is the device referenced in the premarket notification, by the manufacturer of the

"new" device, as the comparison device to comply with the 510(k) regulation.

PREDICTIVE VALUE OF A NEGATIVE TEST RESULT [PV(-)] The probability (P) that the subject is free of disease/state of interest (D-), given that the test is negative (T-), i.e., $P(D-|T-)$. Or, the proportion of individuals who test negative who do not have clinically significant disease/state of interest (or who will not develop it in the future). Predictive values are a function of prevalence of the disease/state of interest in the intended use population and the performance characteristics of the test.

$$PV(-) = P(D-|T-) = \frac{S_2(1 - P)}{(1 - S_1)P + S_2(1 - P)}$$

Where: T = test result, D = disease/state of interest, S_1 = diagnostic sensitivity, S_2 = diagnostic specificity, and P = disease/state of interest prevalence (frequency in the target population).

PREDICTIVE VALUE OF A POSITIVE TEST RESULT [PV(+)] The probability (P) that the subject has the disease/state of interest (D+), given the test is positive (T+), i.e., $P(D+|T+)$. Or, the proportion of individuals who test positive who have clinically significant disease/state of interest (or who will develop it in the future).

$$PV(+) = P(D+|T+) = \frac{S_1P}{S_1P + (1 - S_2)P(1-P)}$$

Where: T = test result, D = disease/state of interest, S_1 = diagnostic sensitivity, S_2 = diagnostic specificity, and P = disease/state of interest prevalence (frequency in the target population).

PREMARKET EVALUATIONS

Presented below is a brief description of the types of applications that are submitted to FDA for the premarket clearance/approval of in-vitro diagnostic devices.

Premarket Notification [referred to as 510(k)]

Section 510(k) of the Federal Food, Drug and Cosmetic Act is the basis for the Premarket Notification, and states that each manufacturer register, prior to introduction into interstate commerce, for the commercial distribution of a Class I or II medical device intended for human use. The 510(k) requires the manufacturer to demonstrate in the premarket notification that the new device is substantially equivalent to a legally marketed device. The Safe Medical Device Act of 1990 also authorizes the FDA to request clinical data as needed to validate that the new device is safe and effective for its intended use.

Premarket Approval [abbreviated as PMA]

Section 515 of the Federal Food, Drug, and Cosmetic Act, requires that manufacturers of Class III devices receive premarket approval by the FDA prior to introducing the device into interstate commerce. A device is categorized as class III if insufficient information exists to determine that general controls are sufficient to provide reasonable assurance of its safety and effectiveness.

Typically, class III devices include: those intended to support or sustain life, those of substantial importance in preventing the impairment of human health, or those devices which may pose a potential risk of illness or injury resulting from their use. The holder of the PMA must provide assurance of the safety and effectiveness of the device in order to gain FDA approval.

PROBE (nucleic acid) Defined strand of nucleic acid used to identify/detect a specific complementary (target) nucleic acid sequence.

QUALITATIVE TEST Yields a binary response, such as plus/minus, disease/non-disease, present/absent, yes/no, etc.

QUALITY ASSURANCE (QA) The practice that encompasses all endeavors, procedures, formats, and activities directed towards ensuring that a specified quality of product is achieved and maintained. This includes the pre-analytic, analytic, and post-analytic phases of a clinical laboratory test system.

In the clinical laboratory, quality assurance includes, but is not limited to, monitoring all the raw materials, supplies, instruments, storage chambers, reaction vessels, procedures, and all else involved in the production of the data reported. (NCCLS EP11-P. 1992;14). QA extends to the laboratory's interactions with and responsibilities to patients, physicians, other laboratories, and other departments of the facility, organization, or institution of which it is a part.

A QA program should: evaluate all established policies and procedures for their effectiveness; identify and correct problems; assure accurate, reliable and prompt test reports; and assure the adequacy and competency of the staff. This encompasses the entire testing process from patient preparation and specimen collection, through test analysis and finally, to test result reporting. (CLIA)

QUALITY CONTROL (QC) An organized system for continuously monitoring the variability of analytical processes performed in the laboratory. (NCCLS EP-11-P)

For moderate or high complexity testing or both, monitoring and evaluating the quality of the analytical testing process of each method to assure the accuracy and reliability

of patient test results and reports is required. The methods must meet the applicable standards in §493.1201 through 493.1221, unless an alternative procedure specified in the manufacturer's protocol is accepted as meeting the CLIA requirements for quality control, or HCFA approves an equivalent procedure as specified in Appendix C of the State Operational Manual (HCFA PUB. 7). (42 CFR §493.1201)

QUANTITATIVE TEST Yields responses that fall on a continuous scale.

RANGE For quantitative claims, the limits within which something varies. (Thiers NCCLS) Range may refer to maximum and minimum possible values, or some particular upper and lower percentiles. It is specific to a defined population. It is also specific to the analytical means by which the limits are ascertained or estimated, such as limits determined by a particular reference method.

REFERENCE METHOD A method that correlates closely to a definitive method/true value in the hands of a series of expert laboratories. (For definitive method, see Gold Standard Method). A reference method has a demonstrated record of transferability and accuracy. A reference method is established by consensus among authorities in a given field.

RELIABILITY The property of an estimate in which it is both precise and unbiased.

REPEATABILITY A reflection of the precision of a measurement. Degree of agreement between successive results obtained under the same conditions of testing (same method, identical test sample, same operator, same apparatus, same laboratory, and over a short time interval).

REPRODUCIBILITY Similar to repeatability except that results are obtained under different (heterogeneous) conditions, e.g., different operators, different equipment, different laboratories, over periods of time. Reproducibility is a reflection of both the precision and accuracy of an estimate.

RESEARCH DEVICE A research device must be labeled: "For Research Use Only. Not for use in diagnostic procedures." The product has no special clinical or diagnostic claim i.e., the sponsor is not making a claim for clinical utility or clinical performance. Consequently, the test results are not used for test reporting or for establishing the performance characteristics of a test.

RECEIVER OPERATOR CHARACTERISTICS (ROC) CURVE A graphic display of the relationship between the sensitivity and specificity of a diagnostic device corresponding to various cut-off values. Traditionally, sensitivity is plotted on the vertical axis and the complement of specificity is plotted on the horizontal axis. The ROC curve can be used to select the best cutoff value for a particular intended use

and a particular target population.

RUN (ANALYTIC) For purposes of QC, an analytic run is an interval of time within which the accuracy and precision of a series of measurements is expected to be stable. Between analytical runs, events may occur causing the measurement process to be susceptible to variations which are important to detect. (Thiers NCCLS)

SMDA '90 The Safe Medical Devices Act of 1990

SAFETY There is reasonable assurance that a device is safe when it can be determined, based on valid scientific evidence, that the probable benefits to health from use of the device for its intended uses and conditions of use, when accompanied by adequate directions and warnings against unsafe use, outweigh any probable risks. [21 CFR 860.7(d)(1)]

SCREENING TEST Generally used to evaluate the genetic status of asymptomatic individuals who are not at increased risk due to a positive family history.

SEMI-QUANTITATIVE TEST Can yield a limited number of possible responses, though more than two, e.g., a continuous scale broken up into a limited number of intervals.

SENSITIVITY The ability of a device/test under study to give a positive result when the end-point of interest (e.g., disease/genetic state/target sequence) is present.

Analytical sensitivity The ability of an assay to detect a particular target sequence/mutation of interest. The proportion of available targets that are detected by the device, i.e., the probability (P) that the target sequence is detected, given that it is present.

The smallest concentration change that a method is capable of detecting.

Diagnostic sensitivity The probability (P) that the test result is positive (T+), given that the subject being tested is disease/state of interest positive (D+), i.e., $P(T+|D+)$. Or, the ability of a device/test under study to give a positive result for subjects having the disease/state of interest.

Test positivity in disease; true positive ratio; ability of a test to correctly identify disease (NCCLS EP11-P. 1992;15).

Comparative, Co-sensitivity or Relative sensitivity For qualitative and semi-quantitative tests, this refers to a test method's ability to yield a positive result when being compared to another method's positive results. For example,

comparison of ISH to traditional metaphase cytogenetic analysis, PCR vs. Southern blot test results, etc.

SPECIFICITY The ability of a device/test under study to give a negative result when the end-point of interest (e.g., disease/genetic state/target sequence) is not present. Ability to distinguish the target of interest from other sequences in the specimen/genome.

Analytic Specificity The ability of the device to distinguish the target sequence(s)/allele(s)/mutation(s) of interest from other sequence(s)/allele(s)/mutation(s) in the specimen/genome. The extent to which the device/assay responds only to a specified target sequence as distinct from other sequences within the sample/genome.

Freedom from interference by any element or compound [DNA sequence] other than the analyte [target] of interest. Tested DNA sequences other than the target(s) of interest, when present or added to a specimen, will not change the result or analyte concentration as measured by a specific method.

Diagnostic specificity The probability (P) that the test is negative (T-), given that the subject being tested is disease/state of interest free (D-), i.e., $P(T-|D-)$. Or, the ability of the device under study to give a negative result for subjects who do not have the disease/state of interest.

Test negativity in non-disease; true negative ratio; ability of a test to correctly identify non-disease.

Comparative, Co-specificity, or Relative Specificity For qualitative or semi-quantitative tests, this refers to a test method's negative result when being compared to another [imperfect] method's negative results.

Location Specificity Refers to the physical map position (location) of the target sequence of interest; whether it is limited to a specific chromosome region/band/locus/etc.

SPECIMEN A sample or part of a body fluid or tissue taken to show or determine the character of the whole. (Thiers NCCLS)

STANDARD Primary reference material.

STATE OF NATURE The true "state of affairs", usually about the genotype of an individual but may relate to the clinical phenotype, e.g., normal non-carrier vs. carrier vs. affected (clinical phenotype present).

STRINGENCY Refers to the degree to which the hybridization reaction condition favors or disfavors duplex association (hydrogen bonding). Under high stringency conditions, only hybridized strands with strong homology will remain hybridized. Hybridized strands with poor homology will dissociate under low stringency.

TARGET POPULATION The population for whom the device or test is intended.

TARGET SEQUENCE The DNA/RNA sequence to be detected. It is a sequence against which a probe is directed in ISH. In general, the probe and the target have complementary nucleic acid sequences.

TYPE I ERROR The probability of concluding that a null hypothesis is not true when it really is.

TYPE II ERROR The probability of concluding that a null hypothesis is true when it really is not.

VALIDATION Confirmation of results found with one set of data using another comparable but independent set of data.

VERIFICATION (Also see Validation) Under CLIA'88, this refers to testing performed by a laboratory to determine if manufacturer established device performance characteristics are being obtained in the laboratory setting.

GLOSSARY REFERENCES:

1. Thiers RE. Proposed guideline. Nomenclature and definitions for use in the National Reference System for the Clinical Laboratory. Order Code: NRSCL8-0 ISSN 0273-3099 Vol 5 number 21. (NRSCL = National Reference System for the Clinical Laboratory incorporating clinical chemistry and other disciplines. Defines definitive methods, reference methods, and certified reference materials to NRSCL Council as part of National Reference System).
2. Webster's New World Dictionary. Guralnik DB (ed) Simon and Schuster. 2 ed. New York 1986. A Dictionary of Genetics. King RC, Stansfield WD (eds.) Oxford Univ Press. 2nd ed. New York 1990.